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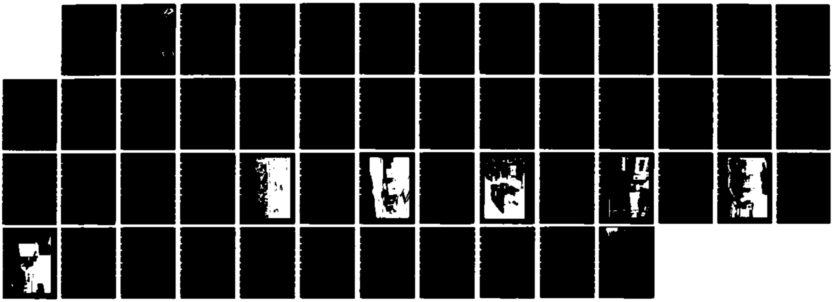

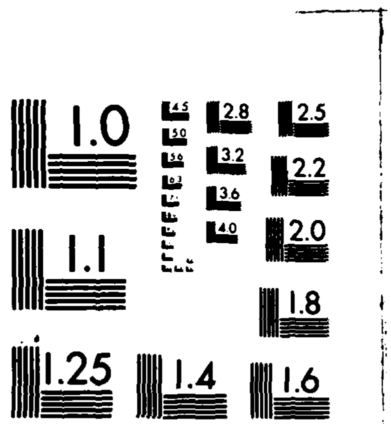

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AN INTEGRATED LIQUID-FROZEN BLOOD BANKING SYSTEM FOR OPERATIONAL FACILITIES

by

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SUMMARY

The second generation Liquid-Frozen Blood Bank System ~~field-tested~~ at the PACOM Blood Program Office, Okinawa, Japan, and at the Fleet Hospital test module deployed at 29 Palms and Bridgeport, California, is capable of providing frozen red cells, frozen platelets, fresh frozen plasma, and cryoprecipitate, to support U. S. Navy and Marine Corps personnel in a war zone.

Red cell concentrates can be frozen after storage at 4 C for 3 to 6 days in CPD or CPDA-1 anticoagulants (non-rejuvenated red cells); red cell concentrates can be rejuvenated and frozen after storage at 4 C for 22 to 25 days in CPD and for 35 days in CPDA-1 (outdated-rejuvenated red cells). Non-rejuvenated and outdated-rejuvenated red cells are frozen in the polyvinylchloride plastic primary collection bag: non-rejuvenated frozen red cells can be stored at -80 C for at least 10 years; outdated-rejuvenated frozen red cells can be stored at -80 C for at least 4 years. The thawed red blood cells are washed with 1.5 liters of sodium chloride-glucose-phosphate solution. Red cells so preserved exhibit in vitro recovery values of at least 90% of the red cells. When previously frozen red cells are stored at 4 C in a sodium chloride-glucose-phosphate solution for as long as 3 days after washing, they have at least 70% 24-hour posttransfusion survival, normal or slightly impaired oxygen transport function, and minimal hemolysis. Excellent results were achieved when red blood cells frozen with 40% W/V glycerol were shipped by air in the frozen state in dry ice (-79 C), and after thawing and washing in wet ice (4 C).

Platelets can be isolated from 4 to 8 units of whole blood and pooled

or they can be isolated from a normal healthy volunteer by an apheresis procedure using a mechanical cell separating system. The platelets can be frozen with 6% DMSO in a polyvinylchloride plastic bag and stored frozen at -80 C for at least 1 year with satisfactory results.

The second generation Liquid-Frozen Blood Bank System can provide 20% of the total red cells, 100% of the platelets, and 100% of the fresh frozen plasma and cryoprecipitate to support a 250- to 1000-bed hospital.

INTRODUCTION

In 1965 the first frozen blood banks were deployed at the Station Hospital, Naval Support Activity, Danang, Republic of Vietnam, and on the Hospital Ships USS Repose and USS Sanctuary. The frozen red blood cells were used to provide an emergency reserve of universal donor O-positive and O-negative red blood cells when liquid-preserved blood was not available to treat combat casualties.[2,3,9] The frozen blood banking system, as a supplemental source of compatible donor red blood cells, provided an alternative to the walking blood donor approach and eliminated a dependency on the presence of healthy men in a war zone to serve as donors at times when demands for blood were high, and in places where blood collection was difficult. The frozen blood products used in this first generation integrated liquid-frozen blood banking system were safe and therapeutically effective in providing medical care for combat casualties.

The freezing technology used during the Vietnam War was not without problems, the primary ones being the large volumes of solution needed to wash each unit of previously frozen red blood cells (6.8 liters) and the restricted post-wash storage period at 4 C for 24 hours.[6] In addition, there was an in vitro red cell loss of 25-30%, a 25% reduction in red cell potassium associated with the then used agglomeration wash procedure and an excessive supernatant hemoglobin concentration in the red cells immediately after washing.[6]

The Naval Blood Research Laboratory has recently developed a second-generation liquid-frozen blood banking system to provide frozen non-rejuvenated and rejuvenated red cells and frozen platelets as a supplement to liquid-preserved blood.[5-8,10-13] Red cells frozen after storage in citrate-phosphate-dextrose (CPD) at 4 C for 3 to 6 days need not be rejuvenated first because they do not have significantly decreased ATP and 2,3 DPG levels after this period of storage.

Red cells that are stored for their mandatory shelf life are rejuvenated to restore the 2,3 DPG and ATP levels that deteriorate during liquid storage. Only O-positive and O-negative outdated red cells are rejuvenated for freeze-preservation because of their broad application as a supplement to the liquid blood banking system.[5,6,8,10,11] Both non-rejuvenated and rejuvenated red blood cells are frozen with 40% W/V glycerol and stored at -80 C. Platelets are frozen with 6% DMSO and stored at -80 C. Research at the Naval Blood Research Laboratory has led to the simplification of procedures for freezing, thawing and washing non-rejuvenated and rejuvenated red cells[10,11] and for isolating, freezing and washing platelets.[1,7,12,13]

The second generation technology for an integrated liquid-frozen blood banking system has recently been field-tested at a Fleet Hospital established by the U. S. Navy as an acute and tertiary medical facility[14]; this system is expected to provide blood products for 250 to 1,000 patients. It is estimated that red cell requirements for a 250-bed hospital would be 200 units of red cells per day, and for a 1,000-bed hospital 800 units of red cells per day. Of these requirements 20% are expected to be fulfilled by frozen red cells, i.e., 40 to 160 units per day, depending upon bed capacity. Cryopreserved platelets and fresh frozen plasma will be available in quantities estimated at 10% those of red blood cells, i.e., 20 to 80 pools of cryopreserved platelets and 20 to 80 units of fresh frozen plasma per day.

The first field test in July 1980, was under desert--summer conditions at the Marine Corps Air Ground Combat Training Center (MCAGCTC), 29 Palms, California. The second field test in March 1981, was under mountain--winter conditions at the Marine Corps Mountain Warfare Training Center (MCMWTC) at Pickel Meadows near Bridgeport, California. An integrated liquid-frozen blood bank system has also been field-tested at the Pacific (PACOM) Blood Program Office at the U. S. Naval Regional Medical Center, Okinawa, Japan, to determine the feasibility of deploying this technology at a fixed-base medical facility. The PACOM Blood Program Office is responsible for providing blood and blood products for the operational needs of the military in the Pacific and Southeast Asia.

The purpose of the field tests at these sites was to examine the technical expertise of military and civilian personnel in operational areas in the use of the special software and hardware necessary for an integrated liquid-frozen blood banking system and to evaluate the safety and efficacy of the blood products. The results of the field testing of the second generation liquid-frozen blood bank system are covered in this report.

MATERIALS AND METHODS

The structural units at the Fleet Hospital housing the blood banks were expandable International Standardization Organization (ISO) containers of the 2:1 type manufactured by the Brunswick Company. These containers were of steel construction, and when fully expanded had internal dimensions of 14' X 19' (266 square feet) with a 7-1/2' ceiling. Electrical, plumbing and ventilation systems for the two modules were designed specifically by the Civil Engineer Support Office, Port Hueneme, California and installed by U. S. Navy Seabees at the Naval Construction Battalion Center, Port Hueneme, California. On-site assembly of the two modules within the Fleet Hospital at the MCAGCTC, 29 Palms, California and the MCMWTC, Bridgeport, California, was performed by Regular and Reserve U. S. Navy Seabee units (Figures 1 and 2). The two modules were assembled as adjoining structures, with entry only to the liquid blood bank module off one of the two main corridors of the Medical/Surgical core area.[14] The frozen blood module could be entered only through the liquid module.

FIG. 1
FIG. 2

The initial study at 29 Palms, California was a limited one, primarily to determine whether the basic equipment could function in a temperature-controlled structure in a desert-summer environment. The second test at Bridgeport, California in a mountain-winter environment was more extensive, with additional equipment to determine whether high-quality high-volume blood component processing could be performed in the liquid and freezing modules.

Major equipment of the liquid blood bank included:

2 - Jewett Model BR-2 blood bank refrigerators

1 - Jewett Model BBR-37 blood bank refrigerator

1 - Jewett Model CFF-1 -30 C blood plasma freezer

1 - 3.9 cubic foot reagent refrigerator

1 - Sorvall GLC-2B table-top centrifuge

1 - Sorvall CW-1 AF-2 automated cell washer

2 - Microscopes

2 - 37 C heating blocks

3 - Office tables - 60' X 34"

1 - 37 C Circulating water bath

This equipment had the storage capacity for approximately 900 units of red blood cells (human) and 60 units of fresh frozen plasma (single human donor). Supplies were available for complete crossmatching, and evaluation of transfusion problems, including atypical antibody identification and quality control. The basic procedures utilized in the liquid blood bank were those detailed in the Technical Manual, American Association of Blood Banks.[4]

Equipment of the frozen blood component module included:

2 - -80 C Harris mechanical freezers

4 - Haemonetics 115 red cell processors

2 - Modified Eberbach shakers

2 - Sorvall RC-3 refrigerated centrifuges

1 - Large Blue-M circulating water bath

3 - Office tables - 60" X 34"

One air-cooled -80 C mechanical freezer (70" L, 28" W, 46" H) had a capacity to store 280 units of red blood cells; four air-cooled -80 C mechanical freezers had a capacity to store 1,000 units of red cells and 100 units of pooled cryopreserved platelets.

The equipment evaluated at the PACOM Blood Program Office, Okinawa, Japan, was similar to that utilized at the Fleet Hospital test module deployed at 29 Palms, California and Bridgeport, California.

The hardware and software and solutions required for biochemical modification (rejuvenation), glycerolization, freezing, and deglycerolization of red cells were provided by the Naval Blood Research Laboratory, Boston, Massachusetts (Figures 3-5). These processes have been described in detail in earlier publications.[5-8,10,11] FIGS. 3-5

The personnel who were assigned to the PACOM Blood Program Office and to the Frozen Blood Bank Module at the Fleet Hospital deployed at 29 Palms and at Bridgeport, California, were provided with copies of Standard Operating Procedures (SOP), prepared by the Naval Blood Research Laboratory, Boston University School of Medicine. The first SOP outlined the processes for freezing non-rejuvenated red blood cells. The second SOP outlined the processes for freezing outdated-rejuvenated red blood cells after storage with a hematocrit value of 80 $\pm$ 5 V% at 4 C in CPD for 22 to 25 days, or after storage in CPDA-1 for 35 days. The stored red cells are rejuvenated with the PIPA solution (containing pyruvate, inosine, phosphate and adenine) and frozen with 40% W/V glycerol in the primary bag of a polyvinylchloride (PVC) multiple-bag collection system. Details of the SOP are briefly outlined below.

Biochemical Modification (Rejuvenation)

For the Fleet Hospital studies at Bridgeport, California, 80 units of human red blood cells were drawn and concentrated at NRMC, Camp Pendleton, California or NRMC, San Diego, California. Each 450 ml unit was drawn into the 800 ml primary bag of a PVC multiple-bag collection system having three integrally attached transfer packs (quadruple blood pack, Code 4R1242, Fenwal Laboratories). Citrate-phosphate-dextrose (CPD) was used as the anticoagulant. Each unit was shipped in the primary bag with attached packs in standard wet ice containers to the Fleet Hospital deployed at Bridgeport, California, where it was maintained at 4 C in the liquid blood bank for a period of 22-30 days before rejuvenation (expiration date 21 days).

The first step in the process of rejuvenating the outdated red cells involved the addition of 50 ml of the PIPA solution (pyruvate, inosine, phosphate and adenine).[11] The solution was added to the red blood cells in the primary bag using a special harness and the adaptor port on the tubing connecting the primary bag to an integrally attached transfer pack. The primary bag (containing the red blood cells and PIPA solution) was placed in a double plastic overwrap which was sealed and placed in a 37 C water bath for 60 minutes of incubation. The rejuvenated red blood cells were frozen with 40% W/V glycerol as described below.

Glycerolization and Freezing

Both the rejuvenated and non-rejuvenated red blood cells were glycerolized to a final 40% W/V concentration of glycerol. Three aliquots of glycerol were added to the red blood cells in the primary bag with a short equilibration period between each addition. Mixing on the modified Eberbach shaker was employed during addition of the first two aliquots of glycerol, and manual agitation was used for the third aliquot. Next, the red blood cells were concentrated by centrifugation in the primary bag, and the supernatant glycerol solution and rejuvenation solution were removed into one of the attached transfer packs for discard.[11]

A label was placed on the primary bag to indicate "Outdated-Rejuvenated Frozen Red Cells (Human)". The bag was placed in a plastic overwrap, sealed, and placed in a cardboard container (7" long X 5" wide X 2" thick). The cardboard container was placed directly in the -80 C freezer.

The process of rejuvenation, glycerolization, and freezing took less than 4 hours. The final glycerol concentration was 40% W/V, and the hematocrit was 65 $\pm$ 5%. Frozen rejuvenated red blood cells can be stored at -80 C for at least 4 years.[10]

Thawing and Deglycerolization

The cardboard container with the plastic-wrapped primary bag containing the frozen red cells was removed from the -80 C freezer. The plastic-wrapped primary bag was removed from the cardboard container and placed directly into a 42 C circulating water bath for 10 to 15 minutes or until completely liquid, with a temperature between 20 and 25 C, after which the protective plastic overwrap was removed.

The Haemonetics 115 red cell processor was used for red cell washing to remove residual amounts of PIPA and glycerol solutions. Initially, a 50 ml aliquot of hypertonic 12% sodium chloride was added to the thawed red cells in the primary bag with agitation on the instrument shaking platform; this was followed by a 2-minute equilibration period without agitation. Next, two aliquots of 0.9% sodium chloride-0.2% glucose-40 mg% inorganic phosphorus with a pH of 6.8 were added to the red cells, again utilizing the shaking platform to ensure adequate mixing. Each addition was followed by 2 minutes of equilibration without agitation.

After the last period of equilibration, the primary bag containing the diluted red cells was suspended in an inverted position on a support hook above the Haemonetics Blood Processor 115 and were allowed to flow into the spinning centrifuge bowl. At the first appearance of waste overflow from the centrifuge bowl, flow of the sodium chloride-glucose-phosphate wash solution was initiated, the flow continuing simultaneously with the remaining red cells entering the bowl until a total volume of 1.5 liters of wash solution was reached. The deglycerolized red cells were expressed from the bowl into a 600 ml plastic bag; the bag contained an integrally attached empty 300 ml transfer pack for subsequent collection of the supernatant solution from the red cells prior to transfusion. A label to indicate "Red Blood Cells (Human), Deglycerolized" was affixed to the 600 ml plastic bag. Washed red cells can be stored at 4 C for 72 hours before transfusion, and the supernatant is removed prior to transfusion.[11]

The time required for processing a unit of red blood cells from the frozen state to a unit ready for transfusion is approximately 40 minutes. Two units of red cells were washed routinely through the same disposable centrifuge bowl using the dry quadruple plastic bag system to collect and store the washed red cells; this can be done only if the two units are transfused to the same patient. In some instances, four units were washed in the same bowl to determine whether this could be done safely in emergency situations.

Studies were also done to determine the effects of transporting frozen rejuvenated red cells in dry ice to a frozen blood module. Dry ice in standard government styrofoam containers (NSN 8115-00-935-9761) normally used for wet ice shipping (19" X 18" X 16") maintained temperatures of -65 C for a 72-hour period (Figure 6). Outdated-rejuvenated frozen red cells were shipped in the frozen state in dry ice (-79 C) from the Naval Blood Research Laboratory, Boston, MA, to the PACOM Blood Program Office, Okinawa, 29 Palms, California, and Bridgeport, California, where they were washed and shipped in wet ice (4 C) back to the Naval Blood Research Laboratory, Boston, Massachusetts. FIG. 6

To evaluate the quality of the cryopreserved red blood cells, measurements were made of freeze-thaw-wash recovery of the red cells, the levels of red cell ATP, 2,3 DPG, and P_{50}, and of the levels of supernatant hemoglobin and extracellular potassium during storage at 4 C in the sodium chloride-glucose-phosphate solution for up to 96 hours.[5,10,11] Samples obtained from the units of washed red cells were cultured on blood agar and in thioglycollate broth and stored at 37 C for 1 week.[5,10,11] In addition, the survival of 10 ml aliquots of autologous red cells was studied in 3 normal volunteers: after 3 days of storage in sodium chloride-glucose-phosphate solution, a 10 ml aliquot from each unit was autotransfused into the original donor. The ^{51}Cr 24-hour posttransfusion survival was measured, and the index of therapeutic effectiveness was calculated from the in vitro recovery of red cells multiplied by the 24-hour posttransfusion survival value.[5,10,11]

Platelet Cryopreservation

Studies were performed to evaluate freeze-preservation of human platelets using 6% DMSO. The platelets were frozen in PVC plastic bags, and the thawed platelets were washed with a solution containing 0.9% sodium chloride-0.2% glucose-40 mg% inorganic phosphorus, pH 5.0.[1]

A 450 ml volume of blood was collected in the CPD or CPDA-1 anticoagulant in the primary bag of the multiple-bag collection system. The platelet concentrate was prepared in one of the integrally attached transfer packs.

The 800 ml primary collection bag of the multiple-bag collection system, with a special adaptor port on the tubing connecting the primary bag to the transfer pack, was used for platelet pooling and freezing. Before the platelets were pooled in the 800 ml primary bag, the CPD anticoagulant was removed into one of three empty transfer packs and the transfer pack was detached and discarded.

Using an AE-7 connector plastic tubing with a stylette on each end, each of 3 to 8 units of ABO and Rh identical platelet concentrates was added to the 800 ml PVC plastic bag as follows: one stylette was inserted into the transfer pack containing the platelet concentrate and the other into the adaptor port on the tubing connecting the 800 ml primary bag to the transfer packs; the same stylette was used to add all the units to the pool in the 800 ml bag. Using the needle site of the 800 ml bag, a

30 to 50 ml volume of 27% DMSO in 0.9% sodium chloride was added to the pool of ABO and Rh identical platelet concentrates to a final DMSO concentration of 6%.

The 800 ml primary bag, containing 100 to 250 ml of platelet-DMSO mixture, with two empty 300 ml transfer packs integrally attached,was placed in an aluminum container for freezing. A 50 ml volume of plasma, taken from one of the units before pooling and transferred to a 150 ml transfer pack, was stored in the same aluminum container alongside the platelets for subsequent use as the resuspension medium. The aluminum container was placed in a -80 C freezer.

In another study of normal healthy volunteers, platelets were isolated using the Haemonetics Blood Processor 30 cell separating machine, with acid-citrate-dextrose (ACD, NIH, Formula A) being used as the anti-coagulant.[12] Six to 8 units of platelets were isolated from the blood of the donor by apheresis into a 600 ml transfer pack. These platelets contained some red blood cells. The red blood cells were concentrated by centrifugation at 800 RPM (160 X g) for 10 minutes. Using an AE-7 connector, with one stylette in the 600 ml transfer pack and the other stylette into the adaptor port attachment of the primary bag, platelet-rich plasma was transferred to the 800 ml primary bag of the multiple-bag collection system from which the anticoagulant had been removed. A 50 ml volume of plasma was collected from each donor for subsequent use as a resuspension medium.

The cryoprotectant, DMSO, was added to the pooled platelet concentrates in the 800 ml bag, and the platelets were frozen at 1-2 C per minute by storage in a -80 C mechanical freezer. The 50 ml volume of plasma collected from each donor was frozen and stored at -80 C in the same aluminum container as the platelets.

The platelets used in this study were collected and frozen at the Naval Blood Research Laboratory, Boston, Massachusetts, and shipped in dry ice to Bridgeport, California. At Bridgeport, the platelets were thawed in a 42 C water bath in about 5 minutes, and then diluted with 250 ml of 0.9% sodium chloride-0.2% glucose-40 mg% inorganic phosphorus, pH 5.0; they were concentrated by centrifugation at 4160 RPM (4500 X g) for 5 minutes, and the supernatant solution was decanted into the two empty integrally attached transfer packs for discard. The 50 ml of plasma frozen alongside the platelet concentrate was thawed and used as the resuspension medium for the washed platelets.

After thawing and washing, the platelets were shipped by air in wet ice (4 C) back to the Naval Blood Research Laboratory. These included: 3 platelet pools with each pool containing 4 units of platelet concentrates from the blood of ABO and Rh identical donors, and one unit of apheresed platelets containing 6 units of platelets isolated from a single donor.

As one part of the study at Okinawa, Japan, 10 healthy donors were plateletpheresed using the Haemonetics Blood Processor 30. The platelets were frozen with 6% DMSO in an 800 ml PVC plastic bag. Prior to freezing, a sample of the DMSO-platelet concentrate mixture was fixed in 1% glutaraldehyde in phosphate-buffered saline: 0.5 ml of the DMSO-platelet mixture was diluted with 4.5 ml of 1% glutaraldehyde in phosphate-buffered saline (PBS). The weight of the DMSO-platelet mixture was recorded so that the volume of platelet-DMSO mixture frozen could be determined: the number of platelets frozen was estimated from the platelet count multiplied by the volume of platelets frozen.

Pools of platelets were also processed as part of this study. Of 100 units of blood collected, 70 units were collected in the CPD anticoagulant, and 30 units in CPDA-1. A platelet concentrate was prepared from each unit, and each platelet concentrate was stored in 20 ml of plasma undisturbed at room temperature for 1 hour before resuspension. Platelets from ABO identical donors were pooled. The platelet pool was frozen with 6% DMSO in an 800 ml PVC plastic bag. Before cryopreservation, a sample of the DMSO-platelet mixture was withdrawn and fixed in 1% glutaraldehyde in PBS, and the weight was recorded.

The frozen apheresed units of platelets and the two frozen platelet pools each containing platelets from 4 to 6 units of blood, were thawed and washed in Okinawa and shipped in wet ice (4 C) to the Naval Blood Research Laboratory. In addition, 8 frozen apheresed units of platelets and 16 units of pooled platelets, each containing 3 to 8 units of ABO and Rh identical platelets, were shipped frozen in dry ice (-79 C) in cardboard boxes lined with styrofoam to the Naval Blood Research Laboratory, where the frozen platelets were thawed, washed and resuspended. Samples were obtained after thawing and after washing and resuspension to measure the DMSO level and to fix the platelets in 1% glutaraldehyde in PBS.

During the Bridgeport deployment, frozen platelets were thawed, washed, resuspended, and shipped in wet ice (4 C) to the Naval Blood Research Laboratory, Boston, Massachusetts, where they were cultured on blood agar and in thioglycollate broth for 1 week at 37 C. Cultures were also made of frozen platelets that were thawed, washed, and resuspended at the Naval Blood Research Laboratory, Boston, Massachusetts.

The freeze-thaw-wash recovery of the platelets was calculated from the platelet count on the sample of DMSO-platelet mixture fixed in 1% glutaraldehyde prior to freezing multiplied by the volume that was frozen and from the platelet count on the sample of washed-resuspended platelets fixed in 1% glutaraldehyde multiplied by the volume of the platelet concentrate. The H4 Coulter Counter was used to make measurements of the population distribution of fragments, platelets, and microaggregates, and of the volume distribution histograms of the 1% glutaraldehyde platelets prior to freezing and after thawing, washing and resuspension. The quality of the cryopreserved platelets was assessed by culture data, by platelet recovery after the freeze-thaw-wash procedure, and by the platelet population and platelet volume distribution histograms.

RESULTS

The military and civilian personnel at the Fleet Hospital at 29 Palms and Bridgeport, California, and at the PACOM Blood Program Office, Okinawa, Japan, were able to follow the Standard Operating Procedure (SOP) prepared by the Naval Blood Research Laboratory, without difficulty, and to use the appropriate hardware and software for processing non-rejuvenated and outdated-rejuvenated frozen red cells, and frozen platelets.

Results of studies at the PACOM Blood Program Office, Okinawa, Japan, on outdated-rejuvenated red cells were similar to results obtained at the Naval Blood Research Laboratory: Measurements of red cell recovery after the freeze-thaw-wash procedure, level of hemolysis during storage at 4 C, and oxygen transport function were similar (Table I). Staphyloccus aureus was cultured from one unit of outdated rejuvenated red cells frozen and washed at Okinawa, Japan, and shipped by air in wet ice (4 C) to the Naval Blood Research Laboratory, where it was stored at 4 C for 1 week and then at room temperature for 4 weeks when the bacterial growth was cultured (Table I). The other units were negative after 1 to 2 weeks of storage at 4 C and for up to 6 weeks at room temperature.

TABLE I

Table II shows the results for frozen outdated-rejuvenated red cells during the 29 Palms study. Some of the units were frozen at 29 Palms and shipped frozen to the Naval Blood Research Laboratory for thawing and washing; other units were frozen at the Naval Blood Research Laboratory

TABLE II

and shipped in the frozen state to 29 Palms for thawing and washing; and other units were frozen, thawed and washed at 29 Palms and shipped in wet ice (4 C) to the Naval Blood Research Laboratory. There were no significant differences among any of the units in freeze-thaw-wash recovery, red cell hemolysis during post-wash storage at 4 C, or oxygen transport function.

TABLE III
TABLE IV

Tables III and IV report data from in vitro studies made at Bridgeport, California, to evaluate the potential for contamination following rejuvenation of outdated red blood cells; the outdated red blood cells were incubated in the rejuvenation solution in a 37 C water bath for 1 hour before glycerolization, freezing, and washing. In one study, 2 units of glycerolized red cells were washed through the same washing bowl (12 units in 6 bowls). In another study 4 units were washed through the same bowl (8 units in 2 bowls). A special dry quadruple plastic bag system was used to recover the 2 units of washed red cells; the supernatant solution from each unit was collected into the two integrally attached empty transfer packs for discard. All 20 units were sterile. Table IV also reports results of a study at Bridgeport in which 2 units of red cells were frozen with 40% W/V glycerol and stored at -20 C, and 4 units were frozen with 40% W/V glycerol and stored at -80 C. Results were not significantly different, although previous data from studies at the Naval Blood Research Laboratory have shown that red cells frozen with 40% W/V glycerol can only be stored for limited periods at -20 C.

TABLE V

Table V reports data from a study in which outdated-rejuvenated red cells were frozen at the Naval Blood Research Laboratory and shipped by air in the frozen state in dry ice (-79 C) to Bridgeport, where they were thawed and washed with 0.9% sodium chloride-0.2% glucose-40 mg% inorganic phosphorus, pH 6.8. The washed red cells were resuspended in the final wash solution and shipped in wet ice (4 C) back to the Naval Blood Research Laboratory, with a total 4 C post-wash storage period of 3 days. Twenty-four-hour posttransfusion survival values were greater than 70%, index of therapeutic effectiveness was 66-71%, oxygen transport function was improved, and only minimal hemolysis was observed (Table V).

The PVC plastic bags used in these studies withstand storage at -80 C and shipment extremely well; the incidence of breakage was less than 3%, much lower than that observed in previous studies in which polyolefin plastic containers were used.

We were also encouraged by our preliminary results with the field-testing of cryopreserved platelets. The platelets were frozen with 6% DMSO in PVC plastic bags and were shipped in aluminum containers in dry ice (-79 C). No contamination was observed in three units of platelets frozen at the Naval Blood Research Laboratory and shipped in dry ice (-79 C) to Bridgeport where they were thawed and washed and shipped back to the Naval Blood Research Laboratory in wet ice (4 C), nor was there any sign of contamination in 4 units of platelets frozen, thawed and washed at Okinawa and shipped in wet ice (4 C) to the Naval Blood Research Laboratory. Platelets frozen at Okinawa and shipped in dry ice (-79 C) to the Naval Blood Research Laboratory where they were thawed and washed, had freeze-thaw-wash recovery values slightly lower than those of washed previously frozen platelets that had not been subjected to shipment in the frozen state.

Platelet counts made by phase microscopy and by the Coulter Counter and the measurement of population and volume distribution histograms in non-fixed and glutaraldehyde-fixed samples of DMSO-treated platelets prior to freezing were not significantly different.

DISCUSSION

Field-testing of the second generation liquid-frozen blood banking system at the PACOM Blood Program Office, Okinawa, Japan, and at the Fleet Hospital deployed at 29 Palms and at Bridgeport, California, demonstrated the feasibility of maintaining a supply of frozen red cells, frozen platelets, fresh frozen plasma, and cryoprecipitate at these sites as an integral part of the hospital's blood bank. Military and civilian personnel at the PACOM Blood Program Office, Okinawa, Japan, and at the Fleet Hospital deployed at 29 Palms and at Bridgeport, California, following protocols prepared at the Naval Blood Research Laboratory, satisfactorily processed rejuvenated red blood cells and frozen platelets.

A red cell rejuvenation process developed at the Naval Blood Research Laboratory, Boston, Massachusetts, is an important aspect of the liquid-frozen blood banking system. By this process, we are able to salvage outdated universal donor O-positive and O-negative red blood cells that would otherwise be discarded: In Vietnam, over 50% of the units of red blood cells reached their outdating period and were discarded before they could be used. A newly designed PVC plastic multiple-bag collection system has simplified the rejuvenation and cryopreservation procedures: component separation, rejuvenation, glycerolization, freezing, and pre-wash dilution all are carried out in this system. Approximately 90% of the red blood cells are recovered after washing as compared with 70-75% with the original method used to freeze red cells in the Vietnam era.[6] The previously frozen red cells can be stored safely at 4 C in a sodium chloride-glucose-phosphate solution for 3 days after washing: 24-hour posttransfusion survivals are at least 70%, oxygen transport function is normal or slightly improved, and hemolysis is minimal. Important also from a logistic viewpoint is the smaller volume of sodium chloride-glucose-phosphate solution used for red cell washing, down from the previously used 6.8 liters to 1.5 liters.

Two units of red cells can be washed in the same disposable bowl in the Haemonetics Blood Processor 115, but only if both units are transfused to the same recipient. Each washed unit is collected in a 600 ml plastic bag of a dry quadruple-bag system; an empty 300 ml bag is integrally attached to each 600 ml bag for collection of the supernatant solution when the red cells are concentrated prior to transfusion. There may be emergency situations in which it would be necessary to wash as many as 4 units in the same disposable bowl, and in our studies we observed no sign of contamination whether 2 or 4 units were washed through the same bowl.

With the simplified technology employed in this study, Navy Corpsmen, with only 4 hours instruction, were able to process largé numbers of high quality frozen red cells within a short period of time. Using a gravity controlled continuous-flow wash system, one technician can operate 3-4 cell washers at once. Additionally, and most important in an operational setting, the instrumentation requires only minimal maintenance and a small amount of space. The modules used in the 29 Palms and Bridgeport deployments can support 8 cell washers (Figure 7), FIG. 7 permitting 2 or 3 technicians to wash 160 units of red cells in a 10-hour work day. The ability to generate this volume of high quality red cells in an operational setting permits storage of a large depot of frozen red cells for use in times of peak red cell utilization.

The studies at the PACOM Blood Program Office at Okinawa, Japan, exhibited the flexibility of the special 800 ml quadruple PVC plastic bag system designed for processing red cells for cryopreservation in adapting to the cryopreservation of pools of platelet concentrates isolated from ABO and Rh identical units of blood. The pooled platelets are frozen with 6% DMSO in an 800 ml plastic bag in an aluminum container in a -80 C freezer. After thawing, the platelets are washed with 250 ml of a solution of 0.9% sodium chloride-0.2% glucose-40 mg% inorganic phosphorus, pH 5.0, to reduce the DMSO concentration to less than 5%. The washed platelets are resuspended in a 50 ml volume of plasma; the plasma sample used as the resuspension medium is taken at the time of platelet collection and frozen and stored in a 150 ml plastic transfer pack in the same aluminum container used for platelet freezing. Frozen units of pooled platelets (from ABO and Rh identical donors) and frozen units of pheresed platelets were sterile and had satisfactory freeze-thaw-wash recovery values after shipment in the frozen state in dry ice (-79 C) from Okinawa, Japan, to the Naval Blood Research Laboratory, Boston, Massachusetts.

In vitro quality control tests included: platelet freeze-thaw-wash recovery, culture data, and population and volume distribution histograms measured on the H4 Coulter Counter after washing and resuspension of the platelets. Limited studies suggest that accurate measurements of platelet counts and platelet population and volume distribution can be made with pre-freeze samples of DMSO-platelet mixture fixed with 1% glutaraldehyde in phosphate-buffered saline solution, but more extensive data are needed. Data are also needed on ^{51}Cr recovery in vivo, lifespan, and function of cryopreserved platelets that have been transported in the frozen state with dry ice.

The studies outlined in this report indicate the feasibility of using a Frozen Blood Bank Module to supplement the liquid blood banking system in an operational setting. At such a facility, frozen outdated-rejuvenated universal donor O-positive and O-negative red cells with a storage capability at -80 C for at least 4 years could furnish 20% of the red cell requirements, and 100% of the platelet needs could be met with cryopreserved platelets with storage at -80 C of at least 1 year.

The second generation Frozen Blood Bank system has proven feasible as a supplement to the liquid blood bank at the Fleet Hospital and at the PACOM Blood Program Office, Okinawa, Japan. Future projects are to study the feasibility of establishing frozen blood banks on LHA, LPH, and Hospital Ships. The following equipment used in the new frozen blood bank module will be evaluated on Navy ships: air-cooled -80 C mechanical freezers, refrigerated centrifuges, red cell washers, modified Eberbach shakers, and water baths. If the equipment can function at sea and if space is adequate, we will evaluate the quality of frozen red cells, frozen platelets, and frozen labile clotting and opsonic plasma proteins prepared at the integrated liquid-frozen blood bank module aboard the LHA, LPH, and Hospital Ships.

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FIGURE 1

Aerial view of the Fleet Hospital deployed at Bridgeport, California.

FIGURE 1

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FIGURE 2

View of the liquid-frozen blood bank system deployed at Bridgeport, California.

FIGURE 2

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FIGURE 3

The interior of the Frozen Blood Bank Module at Bridgeport, California. Four Haemonetics Blood Processor 115's are shown in this view.

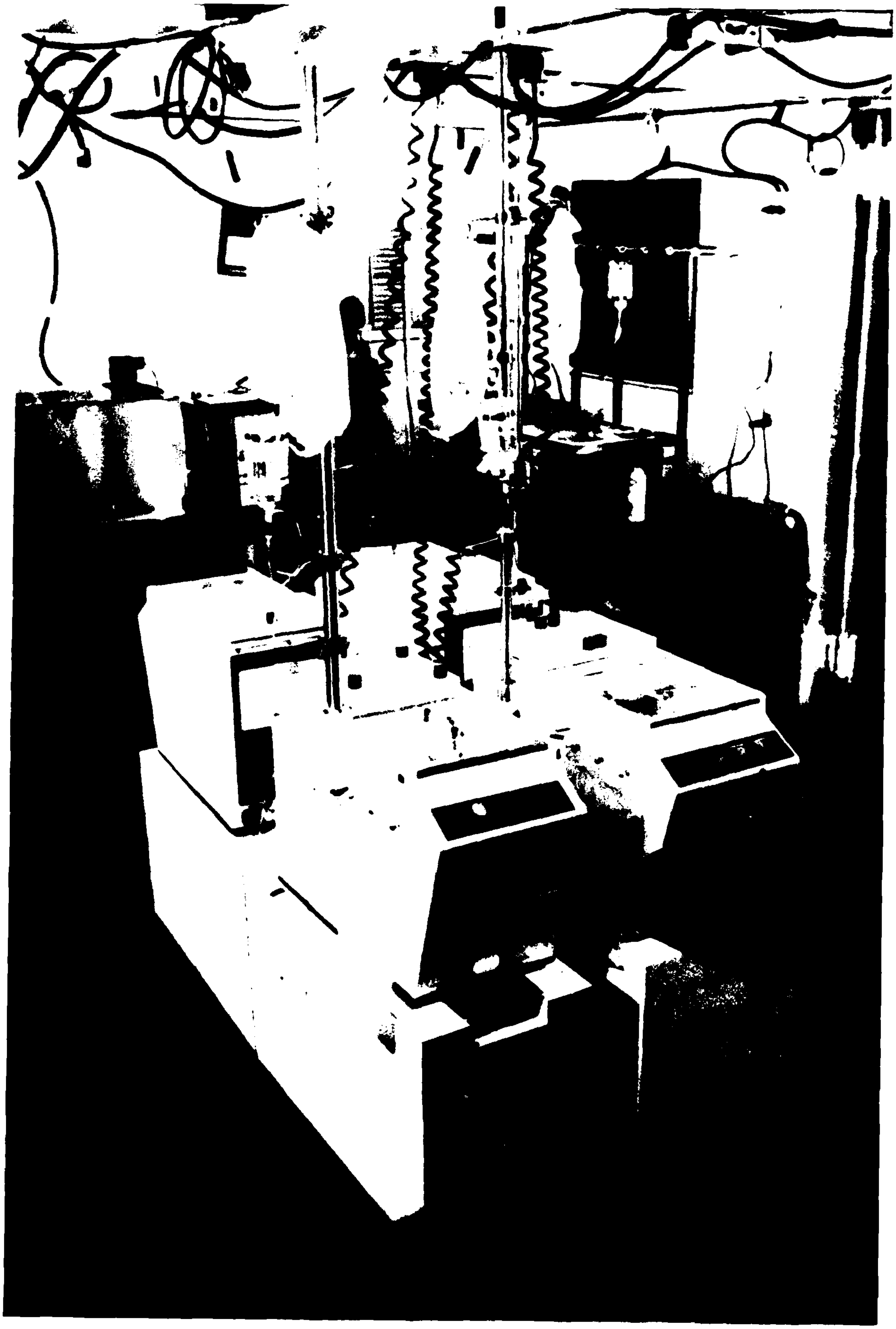

FIGURE 3

FIGURE 4

The interior of the Frozen Blood Bank Module; 2 Haemonetics Blood Processor 115's and 2 air-cooled -80 C mechanical freezers are shown.

FIGURE 4

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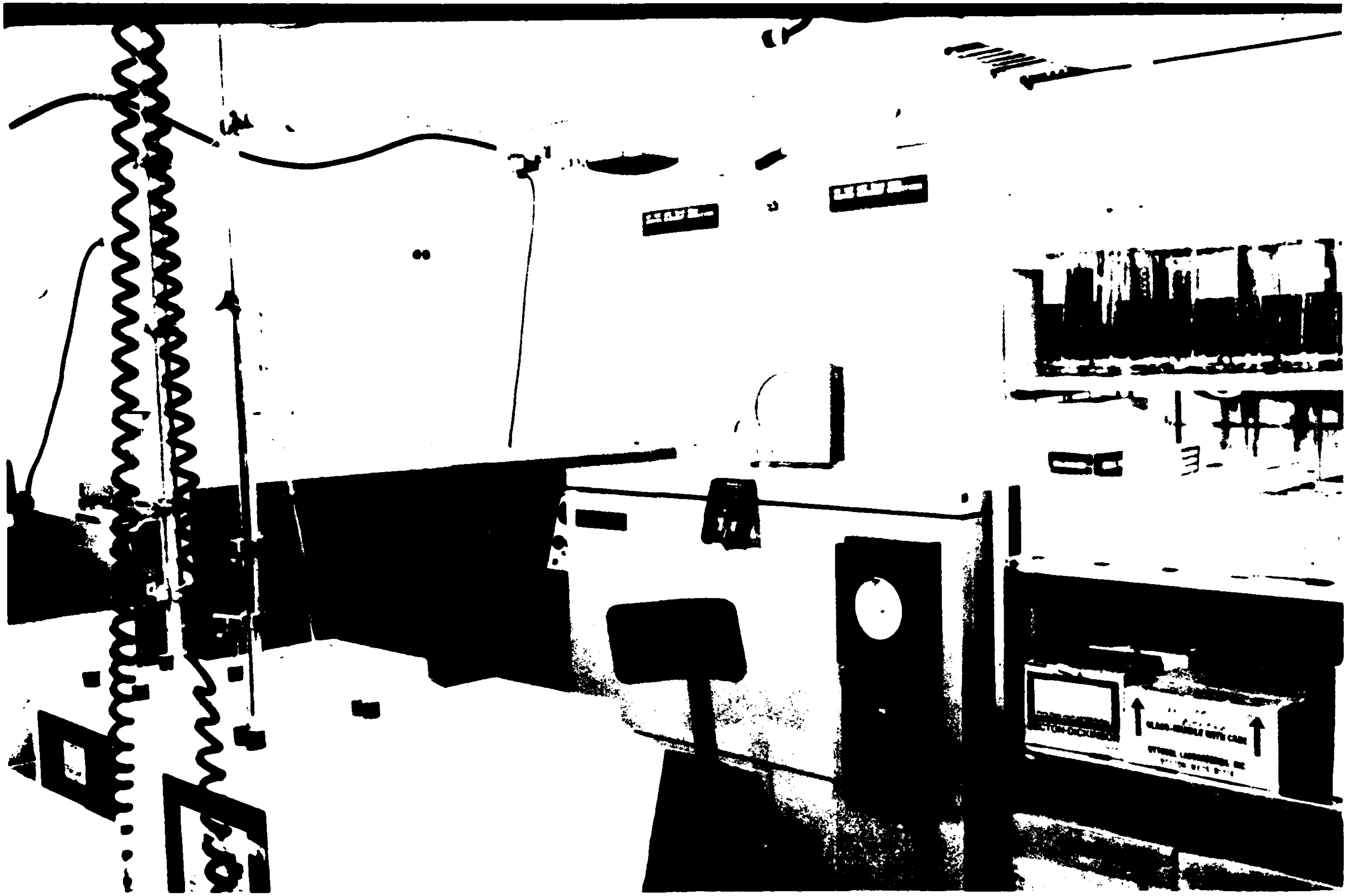

FIGURE 5

The interior of the Frozen Blood Bank Module. Two Haemonetics Blood Processor 115 red cell washers, two air-cooled -80 C mechanical freezers, and the solutions in plastic containers and in bottles used to wash the frozen red blood cells are shown.

FIGURE 5

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FIGURE 6

Shipment of frozen red blood cells with dry ice (-79 C) in a styrofoam insulated cardboard box.

FIGURE 6

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FIGURE 7

Diagram of the Frozen Blood Bank Module designed to fit into an expandable Isocontainer with external dimensions of 20' long, 16' wide, and 8 ' high. The space contains 2 air-cooled -80 C mechanical freezers, 8 Haemonetics Blood Processor 115 red cell washers, 2 refrigerated centrifuges, 2 water baths, and 2 modified Eberbach shakers.

FIGURE 7

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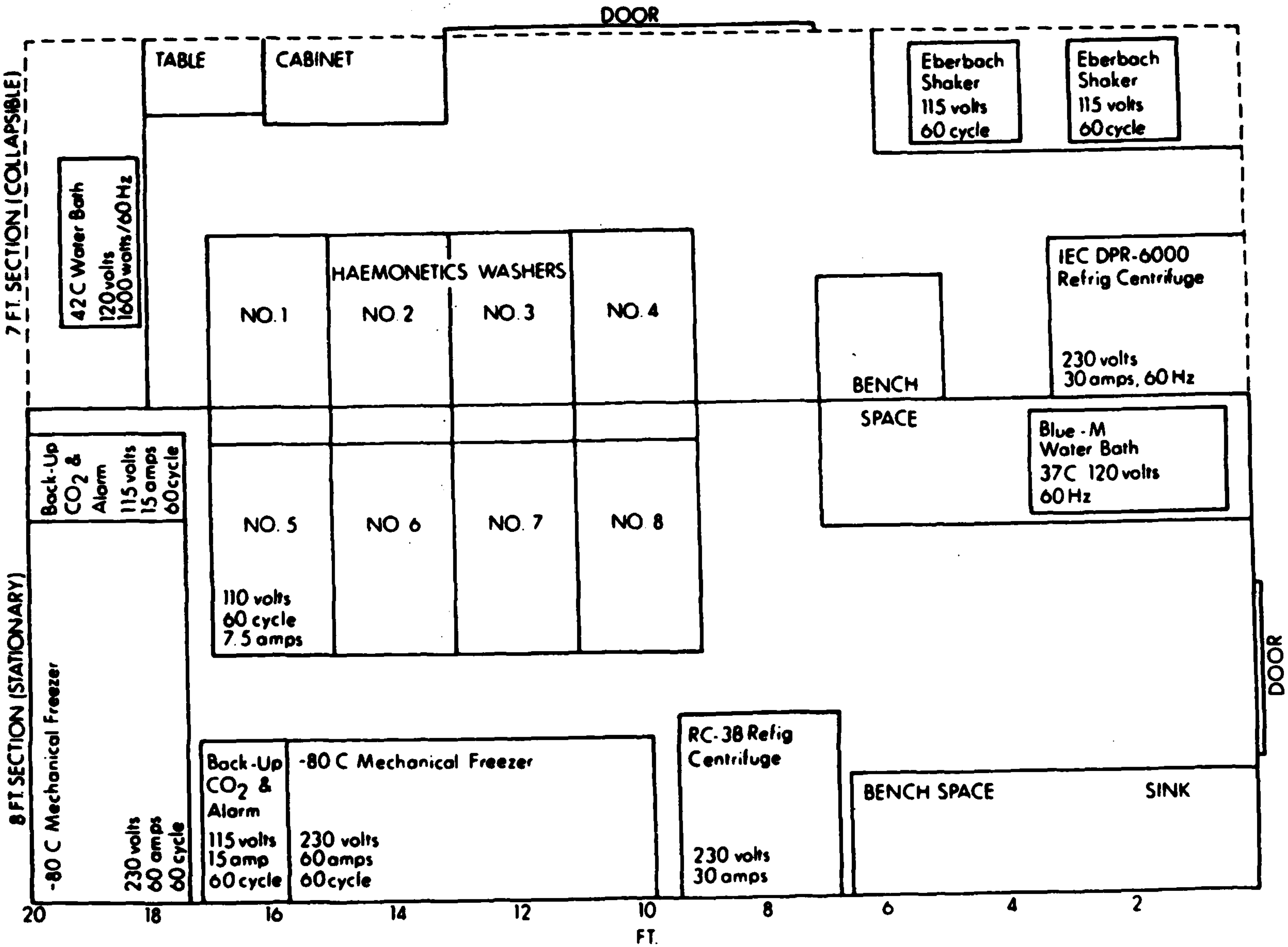

TABLE I

OKINAWA (PACOM) RED CELL STUDY

Red blood cell concentrates with hematocrits of 80 ± 5V% stored at +4 C in CPD for 22 to 25 days; biochemically modified with PIPA, frozen and stored at -80 C with 40% W/V glycerol in the original 800 ml polyvinylchloride plastic collection bag at Okinawa, Japan, then shipped in dry ice (-79 C) to the Naval Blood Research Laboratory, Boston, MA, washed with 0.9% sodium chloride, 0.2% glucose and 40 mg% inorganic phosphorus, pH 6.8 and stored at +4 C for 3 days. In vitro data on these units are tabulated.

WASH METHOD: HAEMONETICS BLOOD PROCESSOR 115
ANTICOAGULANT: CPD
REJUVENATION: PIPA
STORAGE: RBC CONCENTRATE, HCT 80 ± 5 V%
WASH SOLUTION: 0.9% NaCl, 0.2% GLUCOSE, 40 mg% INORGANIC PHOSPHORUS, pH 6.8

	STORAGE DAYS		% RECOVERY		2,3 DPG (uM/g Hb)				ATP (uM/g Hb)				P50 (mm Hg)			
	PRE-FZ	FZN	THAW	WASH	0 HR	24 HR	48 HR	72 HR	0 HR	24 HR	48 HR	72 HR	0 HR	24 HR	48 HR	72 HR
MEAN	23.0	34.2	97.5	91.8	21.2	16.5	15.3	14.5	7.4	5.2	4.7	5.1	38.1	38.5	37.5	36.2
SD	1.5	8.0	1.1	2.2	5.1	2.1	1.7	1.7	1.3	0.4	1.1	0.7	2.2	1.5	2.2	2.3
RANGE	22- 25	18- 38	95.7 98.5	88.6 94.9	12.4- 27.5	14.1- 18.5	13.3- 17.7	12.6- 16.9	5.5- 8.5	4.7- 5.7	4.0- 5.5	4.1- 5.8	35.9- 41.4	36.0- 40.1	35.1- 40.4	33.5- 39.5
N	6	6	6	6	6	6	6	5	6	6	5	6	6	6	5	5

	SUPERNATANT HB (mg%)				EXTRA K^+ (mEq/l)				OSMOLALITY (mOsm/Kg H_2O	HYPO-XANTHINE (uM/ml)	INOSINE (uM/ml)	URIC ACID (mg%)	INORG. PHOS. (mg%)	pH AT 22 C
	0 HR	24 HR	48 HR	72 HR	0 HR	24 HR	48 HR	72 HR	0 HR	0 HR	0 HR	0 HR	0 HR	0 HR
MEAN	44.6	160.1	257.3	383.8	1.0	5.2	8.0	9.8	344	0.246	0.005	0.07	32.6	6.76
SD	10.2	50.4	66.6	109.5	0.2	1.1	1.6	2.1	6	0.060	0.003	0.16	1.7	0.03
RANGE	33.3- 58.7	125.0- 258.0	200.0- 383.0	283.3- 558.0	0.6- 1.2	4.1- 6.6	6.4- 10.2	7.8- 12.3	335 354	0.156- 0.327	0.000- 0.008	0.00- 0.36	30.2- 34.4	6.72- 6.80
N	6	6	6	5	6	6	6	5	6	6	6	6	6	6

All units were tested for bacterial contamination on the day of washing at the Naval Blood Research Laboratory, stored at +4 C for 7 days, then stored at room temperature for up to 6 weeks. Each unit was cultured every 7 days. Bacterial contamination was detected in one of the 6 units studied.

TABLE II

29 PALMS RED CELL STUDY

Red cell concentrates with hematocrits of 80 ± 5 V% stored at 4 C in CPD for 21 days, biochemically modified with PIPA, frozen and stored at -80 C with 40% W/V glycerol in the original 800 ml polyvinylchloride plastic collection bag at 29 Palms, California, then thawed and washed with 1.5 liters of sodium chloride-glucose-phosphate solution, pH 6.8, using the Haemonetics Blood Processor 115, stored at 4 C, and then shipped in wet ice (+4 C) to the Naval Blood Research Laboratory. In vitro data on these units are reported.

WASH METHOD: HAEMONETICS BLOOD PROCESSOR 115
ANTICOAGULANT: CPD
REJUVENATION: PIPA
STORAGE: RBC CONCENTRATE, HCT 80 ± 5 V%
WASH SOLUTION: 0.9% NaCl, 0.2% GLUCOSE, 40 mg% INORGANIC PHOSPHORUS, pH 6.8

	STORAGE (DAYS)		% RECOVERY		2,3 DPG (u mol/g Hb)		ATP (u mol/g Hb)		P50 (mm Hg)		SUPERNATANT HB (mg %)	
	PRE-FZ	FZN	THAW	WASH	48 HR	72 HR	48 HR	72 HR	48 HR	72 HR	48 HR	72 HR
MEAN	21	1.0	98.7	92.3	12.5	9.6	5.5	4.2	35.2	32.1	203.0	207.0
SD	0.0	0.0	0.4	2.4	5.7	4.7	0.5	1.0	7.6	7.4	52.0	69.6
RANGE	---	---	98.4-99.2	89.1-94.5	5.3-19.2	3.3-13.4	5.1-6.4	3.2-5.3	24.1-40.5	21.3-37.9	153.0-273.0	145.0-306.0
N	4	4	4	4	4	4	4	4	4	4	4	4

	EXTRA K^+ (mEq/l)		OSMOLALITY (mOsm/Kg H_2O)	HYPOXANTHINE (uM/ml)	INOSINE (uM/ml)	URIC ACID (mg%)	INORGANIC PHOSPHORUS (mg%)	pH AT 22 C
	48 HR	72 HR	48 HR	48 HR	48 HR	48 HR	48 HR	48 HR
MEAN	4.1	6.2	347	0.370	0.026	0.90	35.9	6.57
SD	0.6	0.3	13	0.16	0.044	0.21	2.4	0.02
RANGE	3.6-4.9	5.8-6.4	334-366	0.24-0.59	0.0-0.092	0.72-1.08	32.8-38.5	6.54-6.59
N	4	4	4	4	4	4	4	4

All units were tested for bacterial contamination upon arrival after 3 days of storage at +4 C at the Naval Blood Research Laboratory, stored at +4 C for 7 to 14 days, then stored at room temperature for up to 6 weeks. Each unit was cultured every 7 days. No bacterial contamination was detected in any of the 4 units studied.

TABLE III

BRIDGEPORT RED CELL STUDY

Red cell concentrates with hematocrits of 80 $\pm$ 5 V% stored at +4 C in CPD for 21 to 28 days, biochemically modified with PIPA, glycerolized but not frozen with 40% W/V glycerol in the original 800 ml polyvinylchloride plastic collection bag at Bridgeport, California, washed with 1.5 liters of sodium chloride-glucose-phosphate solution, pH 6.8, using the Haemonetics Blood Processor 115, stored at +4 C and then shipped in wet ice (+4 C) to the Naval Blood Research Laboratory. In vitro data on these units are reported.

WASH METHOD: HAEMONETICS BLOOD PROCESSOR 115
ANTICOAGULANT: CPD
REJUVENATION: PIPA
STORAGE: RBC CONCENTRATE, HCT 80 $\pm$ 5 V%
WASH SOLUTION: 0.9% NaCl, 0.2% GLUCOSE, 40 mg% INORGANIC PHOSPHORUS, pH 6.8

	TOTAL HB (g)		ESTIMATED RECOVERY	SUPERNATANT HB (mg%)		EXTRA K^+ (mEq/l)		2,3 DPG (uM/g Hb)		ATP (uM/g Hb)		P50 (mm Hg)	
	POST WASH UNIT	WASTE	%	72 HR	96 HR	72 HR	96 HR	72 HR	96 HR	72 HR	96 HR	72 HR	96 HR
MEAN	57.0	4.2	92.5	257	254.3	5.6	6.3	12.2	12.5	3.7	4.4	36.0	34.8
SD	8.4	2.5	4.7	130	122.4	1.6	1.8	3.2	3.2	1.1	1.1	2.1	3.6
RANGE	36.9-	1.9-	80.2-	109-	84.0-	3.8-	3.5-	8.1-	7.5-	2.1-	2.6-	32.4-	27.8-
	68.3	9.1	97.0	503	520.0	9.8	11.6	17.5	18.9	5.9	6.7	39.0	41.0
N	20	20	20	11	19	11	19	11	19	11	19	10	16

	HYPOXANTHINE (uM/ml)		INOSINE (uM/ml)		URIC ACID (mg%)		INORGANIC PHOSPHORUS (mg%)	
	72 HR	96 HR	72 HR	96 HR	72 HR	96 HR	72 HR	96 HR
MEAN	0.685	0.678	0.002	0.001	0.36	0.36	36.0	35.8
SD	0.210	0.21	0.003	0.001	0.16	0.0	1.6	2.8
RANGE	0.077-	0.408-	0.000-	0.000-	0.00-	----	32.4-	32.4-
	1.060	1.072	0.008	0.003	0.72	----	37.4	40.3
N	11	8	11	8	11	8	11	6

Each unit was tested for bacterial contamination upon arrival after 3 days of storage at +4 C at the Naval Blood Research Laboratory, stored at +4 C for 7 to 14 days, then stored at room temperature for up to 6 weeks. Each unit was cultured every 7 days. No bacterial contamination was detected in any of the 20 units studied.

TABLE IV

BRIDGEPORT RED CELL STUDY

Red cell concentrates with hematocrits of 80 ± 5 V% stored at +4 C in CPD for 21 to 28 days, biochemically modified with PIPA, frozen and stored at -80 C with 40% W/V glycerol in the original 800 ml polyvinylchloride plastic collection bag, and shipped from the Naval Blood Research Laboratory, Boston, MA in dry ice (-79 C) to Bridgeport, California. Other red cell concentrates were frozen and stored at -20 C with 40% W/V glycerol in the original 800 ml polyvinylchloride plastic collection bag at Bridgeport, California. Each unit was thawed and washed with 1.5 liters of sodium chloride-glucose-phosphate solution, pH 6.8, using the Haemonetics Blood Processor 115, stored at +4 C, and then shipped in wet ice (+4 C) to the Naval Blood Research Laboratory. In vitro data on these units are reported.

WASH METHOD: HAEMONETICS BLOOD PROCESSOR 115
ANTICOAGULANT: CPD
REJUVENATION: PIPA
STORAGE: RBC CONCENTRATE, HCT 80 ± 5 V%
WASH SOLUTION: 0.9% NaCl, 0.2% GLUCOSE, 40 mg% INORGANIC PHOSPHORUS, pH 6.8

	TOTAL HB (g) POST WASH UNIT	TOTAL HB (g) WASTE	ESTIMATED RECOVERY %	SUPERNATANT HB (mg%) 72 HR	SUPERNATANT HB (mg%) 96 HR	EXTRA K^+ (mEq/l) 72 HR	EXTRA K^+ (mEq/l) 96 HR	2,3 DPG (uM/g Hb) 72 HR	2,3 DPG (uM/g Hb) 96 HR	ATP (uM/g HB) 72 HR	ATP (uM/g HB) 96 HR	P50 (mm Hg) 72 HR	P50 (mm Hg) 96 HR
RBC STORED AT -80 C													
MEAN	54.2	2.5	95.6	210	223	3.9	4.8	10.7	7.7	3.8	3.9	31.0	31.1
SD	1.3	0.0	0.1	56	31	0.2	0.2	2.9	3.2	1.0	1.0	4.4	4.3
RANGE	52.8-	---	95.5-	151-	185-	3.6-	4.5-	7.3-	4.5-	3.1-	3.1-	27.5-	25.0-
	55.8	---	95.7	285	319	4.0	5.0	13.8	11.6	5.2	5.4	37.3	34.3
N	4	4	4	4	4	4	4	4	4	4	4	4	4
RBC STORED AT -20 C													
MEAN	54.9	5.0	91.7	201		6.2		11.6		3.4		37.1	
SD	0.8	1.1	1.7	24		2.6		0.5		0.6		1.1	
RANGE	54.3-	4.2-	90.5-	184-		4.3-		11.2-		3.0-		36.2-	
	55.4	5.8	92.9	218		8.0		11.9		3.8		38.0	
N	2	2	2	2		2		2		2		2	

Each unit was tested for bacterial contamination upon arrival after 3 days of storage at +4 C at the Naval Blood Research Laboratory, stored at +4 C for 7 to 14 days, then stored at room temperature for up to 6 weeks. Each unit was cultured every 7 days. No bacterial contamination was detected in any of the 6 units studied.

TABLE IV (cont.)

RBC STORED AT -80 C

	HYPOXANTHINE (uM/ml) 72 HR	INOSINE (uM/ml) 72 HR	URIC ACID (mg%) 72 HR	INORGANIC PHOSPHORUS (mg%) 72 HR
MEAN	0.339	0.007	0.63	37.6
SD	0.188	0.011	0.34	1.3
RANGE	0.188- 0.612	0.000- 0.024	0.36- 1.08	36.0- 39.2
N	4	4	4	4

RBC STORED AT -20 C

	HYPOXANTHINE (uM/ml) 72 HR	INOSINE (uM/ml) 72 HR	URIC ACID (mg%) 72 HR	INORGANIC PHOSPHORUS (mg%) 72 HR
MEAN	0.376	0.0	0.18	37.6
SD	0.085	0.0	0.25	0.3
RANGE	0.316- 0.436	---	0.00- 0.36	37.4- 37.8
N	2	2	2	2

TABLE V

IN VIVO RED CELL STUDIES

Three units of red blood cell concentrates stored at +4 C in CPD or CPDA-1 for 25 to 35 days, biochemically treated with PIPA, frozen in the original polyvinylchloride plastic bag with 40% W/V glycerol, shipped from the Naval Blood Research Laboratory, Boston, MA by air in dry ice (-79 C) to Bridgeport, California, stored at -80 C, washed, stored in 0.9% NaCl, 0.2% glucose, and 40 mg% inorganic phosphorus, pH 6.8, and shipped in wet ice (+4 C) to the Naval Blood Research Laboratory, Boston, MA by air, stored at +4 C for 3 days, and then a 10 ml aliquot of each unit was autotransfused. The in vitro and in vivo data are reported for each unit.

UNIT #	ANTI-COAGULANT	HCT DURING STORAGE	STORAGE AT +4 C PRE-FREEZE (DAYS)	LENGTH OF FROZEN STORAGE (DAYS) (-80 C)	REJUVENATION SOLUTION	STORAGE AT 4 C AFTER WASHING (DAYS)	IN VITRO RECOVERY OF RBC (%)
AT-782	CPD	88	25	15	PIPA	3	89.1
AT-786	CPD	86	25	9	PIPA	3	93.0
AT-784	CPDA-1	85	25	4	PIPA	3	88.2

UNIT #	24 HR ^{51}Cr SURVIVAL (%)	ITE(%)*	SUPT HB (mg%) 72 HR	K^+ (mEq/l) 72 HR	RBC 2,3 DPG (umol/g Hb) 48 HR	RBC 2,3 DPG (umol/g Hb) 72 HR	RBC ATP (umol/g Hb) 48 HR	RBC ATP (umol/g Hb) 72 HR	RBC P50 (mm Hg) 48 HR	RBC P50 (mm Hg) 72 HR	HYPO-XANTHINE (umol/ml) 72 HR	INOSINE (umol/ml) 72 HR	URIC ACID (mg%) 72 HR
AT-782	74	66	285	7.2	---	21.3	---	5.0	37.3	42.5	0.568	0	1.40
AT-786	72	67	260	6.5	15.25	16.1	5.8	6.5	41.3	31.3	0.720	0	0.36
AT-784	80	71	335	6.6	11.70	11.0	3.5	4.4	39.2	31.5	0.644	0	----

UNIT #	INORGANIC PHOSPHORUS (mg%) 72 HR
AT-782	39.6
AT-786	38.5
AT-784	41.8

*Index of Therapeutic Effectiveness

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